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Efficacies of Cefotaxime and Ceftriaxone in a Mouse Model of Pneumonia Induced by Two Penicillin- and Cephalosporin-Resistant Strains of *Streptococcus pneumoniae*

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We previously demonstrated the efficacy of ceftriaxone (CRO), at 50 mg/kg of body weight every 12 h, against a highly penicillin-resistant (MIC, 4 μg/ml) *Streptococcus pneumoniae* strain with low-level resistance to CRO (MIC, 0.5 μg/ml) in a leukopenic-mouse pneumonia model (P. Moine, E. Vallée, E. Azoulay-Dupuis, P. Bourget, J.-P. Bédos, J. Bauchet, and J.-J. Pocidalo, Antimicrob. Agents Chemother. 38:1953–1958, 1994). In the present study, we assessed the activity of CRO versus those of cefotaxime (CTX) and amoxicillin (AMO) against two highly penicillin- and cephalosporin-resistant *S. pneumoniae* strains (P40422 and P40984) (MICs of 2 and 8 for penicillin, 2 and 4 for AMO, and 4 and 8 for CRO or CTX, respectively). Against both strains, a greater than an 80% cumulative survival rate was observed with CRO at a dose of 100 or 200 mg/kg every 12 h (dose/MIC ratio, 25). With CTX, a high dosage of 400 mg/kg (dose/MIC ratio, 100 or 50) administered every 8 h (TID) was needed to protect 66 and 75% of the animals, respectively, with no statistically significant differences versus CRO. Against the P40422 strain, CRO (100 mg/kg) produced the greatest bactericidal effect, from the 8th to the 24th hour after a single injection (1.8-log-unit reduction over 24 h), and the fastest bacterial pulmonary clearance during treatment; with CTX, only multiple injections at a high dosage, i.e., 400 mg/kg TID, demonstrated a significant bactericidal effect. AMO in a high dosage, 400 mg/kg (dose/MIC ratio, 200) TID, showed good activity only against the P40422 strain. Despite the identical MICs of CTX and CRO, the longer time (3.6 to 4.6 h) that serum CRO concentrations remained above the MICs for the pathogens at a dose of 100 mg/kg resulted in greater efficacy versus CTX against highly penicillin- and cephalosporin-resistant *S. pneumoniae* strains.

Streptococcus pneumoniae is still the organism most frequently recovered in patients with community-acquired pneumonia (16, 29, 36). Until the 1960s, pneumococci were considered invariably susceptible to penicillin. The first penicillin-resistant strains of *S. pneumoniae* were reported in 1967 (24). Since then, penicillin resistance of pneumococci has spread throughout the world, complicating the antimicrobial treatment of community-acquired pneumonia (1, 4, 5, 26, 33). Recently published data suggest that high-dose intravenous penicillin G or amoxicillin (AMO) may be adequate in cases of pneumococcal pneumonia due to strains with penicillin MICs in the 0.12- to 2-μg/ml range (18, 26, 37, 38).

Although broad-spectrum cephalosporins (e.g., cefotaxime [CTX] and ceftriaxone [CRO]), which are characterized by higher levels in serum and lower MICs, have been suggested for treatment of pneumonia due to penicillin-resistant strains of *S. pneumoniae* with penicillin MICs of >2 μg/ml, *S. pneumoniae* clinical isolates with high levels of resistance to cephalosporins (MICs of >1 μg/ml) have been reported since 1992 (17, 25, 32). CRO and CTX have been found effective in treatment of pneumococcal pneumonia when their MICs were ≤2 μg/ml (37). Although an upper limit of 8 μg/ml (18) has been suggested for use of broad-spectrum cephalosporins in treatment of pneumonia due to highly penicillin- and cephalosporin-resistant pneumococci, neither well-documented breakpoints nor clear recommendations are available for the management of pneumonia due to such isolates of pneumococci (10, 18).

CRO is a broad-spectrum cephalosporin (3, 9, 12, 14) with distinct pharmacokinetic characteristics, including a long elimination half-life in serum and lungs and a high level of protein binding (8, 13, 39, 45). The time that a serum antibiotic concentration remained above its MIC for the pathogen (Δ*t*MIC) was the pharmacodynamic parameter most closely correlated with β-lactam efficacy in various models of infection (2, 20). The long elimination half-life of CRO in serum, which results in an extended Δ*t*MIC, is probably related to the high affinity of this antimicrobial agent for serum proteins, with the protein-bound fraction serving as a reservoir (19, 31).

We have previously demonstrated the efficacy of CRO against a strain of *S. pneumoniae* with high-level resistance to penicillin (MIC = 4 μg/ml) but low-level resistance to cephalosporins (MIC = 0.5 μg/ml) in a model of pneumonia in severely immunocompromised mice (31). In a mouse model involving intraperitoneal inoculation of *S. pneumoniae*, CRO demonstrated better activity than CTX or cefuroxime, although their MICs were the same, i.e., 0.02 μg/ml (20).

In this experimental study, we compared the efficacy of AMO and of two broad-spectrum cephalosporins, CTX and CRO, with identical MICs, against two highly penicillin- and cephalosporin-resistant strains of *S. pneumoniae* in a mouse model of acute pneumonia.

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MATERIALS AND METHODS

Challenge organisms. We induced pneumonia with two highly penicillin- and cephalosporin-resistant strains of *S. pneumoniae* recovered from middle-ear fluid: strain P40422 (serotype 23F) and strain P40489 (serotype 14). These strains were provided by P. Geslin of the Centre de Référence du Pneumocoque (Créteil, France).

Animals. Female Swiss mice (body weight, 20 to 22 g) were obtained from Iffa-Credo Laboratories, l'Arbresle, France.

Immunosuppression in mice. Like other penicillin-resistant strains, and because of their capsular type, these penicillin- and cephalosporin-resistant strains were naturally avirulent for immunocompetent mice (7, 11), and induction of pneumonia failed without previous immunosuppression. Therefore, mice were pretreated with daily intraperitoneal injections of cyclophosphamide (Endoxan; Sarget Laboratories, Merignac, France) at a dose of 150 mg/kg of body weight for 3 days, beginning 4 days before the infectious challenge. On the day of infection, peripheral leukocyte counts had fallen from about 7,000 to a mean of 850 cells per mm^3 of blood. Under these conditions, the two low-virulence strains expressed their pathogenicity and invasive properties.

Antibiotics. The drugs used in this study were CRO (Roche Laboratories, Neuilly-sur Seine, France), CTX (Roussel Laboratories, Paris, France), and AMO (Beecham Laboratories, Paris, France). The antibiotics were reconstituted according to the package instructions, and the final concentrations were obtained by dilution in sterile water.

In vitro studies. MICs and MBCs were determined by the tube dilution method (35) in Mueller-Hinton infusion broth (Diagnostic Pasteur, Marnes-la-Coquette, France) supplemented with 5% filtered horse serum. Each tube contained a dilution of antibiotic (which had been serially diluted twofold) and had a final bacterial density of 10^6 CFU/ml. Tubes were incubated aerobically for 18 h at 37°C in 10% CO_2–air and then read visually. The MIC was defined as the lowest concentration of antibiotic at which no growth was visible to the naked eye. For MBC determination, 0.01-ml aliquots from all tubes with no visible growth and the last turbid tube were plated onto Columbia agar supplemented with 5% sheep blood (Bio-Mérieux, Lyon, France). The plates were incubated overnight at 37°C in 10% CO_2–air, and the MBC was defined as the lowest concentration of antibiotic that killed ≥99.9% of the original inoculum.

Experimental pneumococcal pneumonia in mice. Leukopenic Swiss mice were infected by peroral tracheal instillation as described elsewhere (6). A volume of 40 μl of exponential-growth-phase bacterial suspension (10^7 CFU per mouse) was delivered directly to the lower respiratory tract. Untreated leukopenic mice developed acute pneumonia, became bacteremic within 4 h after infection, and died within 3 to 4 days, depending on the invasiveness of the strain (with a mortality peak on day 2 or 3).

Treatment regimens. Treatment schedules were as follows. AMO and CTX were administered at 8-h intervals with a total of nine injections, and CRO was given at 12-h intervals with a total of six injections. The dose of each antibiotic varied with the infective strain. Mice infected with strain P40422 were treated with AMO or CTX at 200 or 400 mg/kg or with CRO at 100 mg/kg. Mice infected with P40984 were treated with AMO at 200 or 400 mg/kg, CTX at 400 mg/kg, or CRO at 100 or 200 mg/kg. Each dose was administered subcutaneously (s.c.) in 0.5 ml of sterile water. Control animals received the same volume of isotonic saline.

Bactericidal activity in vivo. The study drugs were assessed for their ability to eliminate bacteria from the lungs. Antimicrobial administration was initiated 3 h after infection and consisted either of a single dose of each antibiotic or a complete course at various dosages. The total number of CFUs recovered from whole-lung homogenates was determined 1, 4, 8, and 24 h after a single injection or 32, 62, and 86 h after the first injection during complete courses (i.e., 24 h after treatment completion for the last sample). Mice were killed by CO_2 asphyxiation and exsanguinated by cardiac puncture. The blood was used for cultures, and the lungs were removed and homogenized in 1 ml of saline. Serial 10-fold dilutions of the homogenates were plated onto Columbia agar supplemented with 5% sterile sheep blood. Each experiment was repeated at least twice. Results were expressed as the mean $\log_{10}$ CFU per milliliter of lung homogenate ± the standard deviation for groups of three mice. The lower limit of detection was 2 $\log_{10}$ CFU per lung, which corresponded to the weakest dilution of tissue homogenates (10^{-1}), avoiding significant drug carryover with control inocula.

Survival studies. Treatment was initiated s.c. 3 h after the bacterial challenge. A total of 15 animals were used per treatment group, and in each experiment the animals were infected simultaneously. Survival rates were recorded daily for 14 days, and cumulative survival rates were compared.

Pharmacokinetic studies. The pharmacokinetic profiles of AMO, CTX, and CRO were examined by bioassay. Concentrations in lungs and sera were determined after a single subcutaneous injection of each antibiotic at various doses: AMO, 200 and 400 mg/kg; CTX, 200 and 400 mg/kg; and CRO, 100 mg/kg. Three animals per group were killed with CO_2 and exsanguinated by intracardiac puncture 0.5, 1, 2, 4, 6, and 8 h following drug administration. Blood samples were centrifuged to isolate serum, which was pooled and frozen at −80°C until assay. The lungs were removed from exsanguinated mice, washed in sterile sodium chloride solution, and homogenized in 1 ml of phosphate buffer solution (pH 6.8). Homogenates were centrifuged, and supernatants were used for assays. The bioactivity in specimens was determined by the agar well diffusion method with *Sarcina lutea* ATCC 9341 and *Escherichia coli* ATCC 39118 as the reference organisms for AMO and for CTX and CRO, respectively. The test media used were Antibiotic Medium 1 for AMO and Antibiotic Medium 2 for CTX and CRO (Difco Laboratories, Detroit, Mich.). Standard curves were produced with solutions of each antibiotic in phosphate buffer, pH 6.8, for serum and tissue, in order to evaluate the active fractions of the antibiotics. Concentrations were determined by averaging diameters from three replicates and comparing the results to a standard curve. Results were expressed as micrograms of drug per milliliter of blood or per gram of lung tissue. The standard curves were linear from 0.125 to 32 μg/ml, and the lower limit of sensitivity was 0.1 μg/ml of specimen. The coefficient of between- and within-day variation for replicates ($n = 5$) was ≤7.5% at 0.5, 1, 7.5, and 20 μg/ml.

Pharmacokinetic analysis. Plasma concentration-time curves were fitted to one- or two-compartment open models according to the curves of plotted data, and parameters were estimated by standard methods (21): C_{max} is the maximal concentration observed; $t_{1/2\beta}$ is the terminal elimination half-life calculated by linear least-squares regression for the log-linear terminal elimination phase; ΔtMIC is the time during which concentrations exceed the MIC for test pathogens; and AUC_{0-24} is the area under the concentration-time curve from 0 to 24 h, calculated by using the trapezoidal rule.

Statistical analysis. Analysis of variance was used to compare intergroup differences in bacterial counts. Survival rate data were analyzed by the unpaired *t* test. *P* values of 0.05 or less with the two tests were considered statistically significant.

TABLE 1. Antimicrobial susceptibilities of *S. pneumoniae* challenge strains to study drugs

Study drug	MIC/MBC (μg/ml) for strain:	
	P40422	P40984
Penicillin	2/4	8/8
AMO	2/4	4/8
CTX	4/4	8/16
CRO	4/4	8/16

RESULTS

In vitro microbiological data. The properties of the two strains are shown in Table 1. Both strains were highly resistant to penicillin, AMO, CTX, and CRO. Cephalosporins were less active than penicillin or AMO (Table 1).

Bacterial clearance from lungs in experimental pneumonia. (i) Pulmonary and blood clearance in leukopenic mice infected with P40422 after a single drug injection. Lung and blood clearance values for P40422-infected mice given one injection of AMO, CTX, or CRO are shown in Table 2. No early bactericidal effect was seen in any of the treatment groups. After a single injection, at no time were statistically significant differences in bacterial counts noted between controls and mice treated with AMO at 200 mg/kg. When the dose was increased twofold (to 400 mg of AMO per kg), bacterial killing was significant 4 and 8 h after the injection. With CTX, bacterial counts decreased to a significant level only 8 h after injection of 200 or 400 mg/kg, without significant differences between the two doses. Intrapulmonary killing of *S. pneumoniae* P40422 was significantly greater at the eighth hour with CRO at 100 mg/kg compared with the control group and with the groups treated with AMO at 200 mg/kg, CTX at 200 mg/kg, or CTX at 400 mg/kg. There was no significant difference between AMO at 400 mg/kg and CRO at 100 mg/kg in intrapulmonary killing until the 24th hour after a single injection, when CRO showed a prolonged antibacterial effect contrasting with regrowth in AMO-treated animals. Bacteria were found in the blood of all controls starting at the fourth hour after infection. Only CRO completely eliminated the bacteria from blood at any time.

(ii) Pulmonary and blood clearance during treatment of leukopenic mice infected with P40422. Kinetic parameters of

TABLE 2. Clearance, after a single s.c. injection of study drugs, of *S. pneumoniae* from lungs and blood of leukopenic Swiss mice infected with strain P40422

Drug and dose (mg/kg)	Clearance after a single s.c. injection as determined at time (h)[a]:							
	1		4		8		24	
	From lung	From blood	From lung	From blood	From lung	From blood	From lung	From blood
None (controls)	7.47 ± 0.17	5/6	7.18 ± 0.28	6/6	7.52 ± 0.27	6/6	8.10 ± 0.15	6/6
AMO, 200	7.22 ± 0.18	2/6	7.56 ± 0.51	2/6	6.70 ± 0.8	1/6	7.70 ± 0.51	2/6
AMO, 400	7.16 ± 0.42	1/6	6.09 ± 0.25[b,c]	0/6	5.80 ± 0.38[b]	0/6	7.20 ± 0.73	1/6
CTX, 200	7.10 ± 0.62	2/6	7.20 ± 0.3	2/6	6.80 ± 0.36[b]	2/6	7.40 ± 0.35	4/6
CTX, 400	7.02 ± 0.18	1/6	6.69 ± 0.35	2/6	6.55 ± 0.33[b]	1/6	7.02 ± 0.15	2/6
CRO, 100	7.20 ± 0.36	0/6	6.80 ± 0.37[b,d]	0/6	5.70 ± 0.38[b,d]	0/6	5.41 ± 0.86[b,c]	0/6

[a] For lung, values are mean $\log_{10}$ CFU per milliliter of lung homogenate mean ± standard deviations ($n = 6$). For blood, values are the number of animals with positive blood cultures/total number of animals.
[b] Value is lower than in the control group ($P = 0.0016$ to $P < 10^{-4}$).
[c] Value is lower than in the other treatment groups ($P = 0.004$ to $P < 10^{-4}$).
[d] Value is lower than in the other treatment groups except AMO at 400 mg/kg ($P = 0.0012$ to $P < 10^{-4}$).

intrapulmonary killing during treatment are given in Table 3. CRO promptly reduced bacterial counts to undetectable levels at the end of treatment without any regrowth. Eight hours after the first injection, bacterial counts were significantly lower in mice treated with CRO at 100 mg/kg twice a day (BID) or with AMO at 400 mg/kg three times a day (TID) than in the control and CTX groups. Elimination of bacteria from lungs was slower with AMO than with CRO: 62 h after the first injection (8 h after eight injections), lung bacterial counts were significantly lower with CRO at 100 mg/kg BID than with AMO at 400 mg/kg TID. Bactericidal activity of CTX was present at 32 and 62 h after the first injection compared with the control group, but killing was less significant and slower than with CRO and AMO. Moreover, there was no significant difference between CTX at 200 mg/kg and CTX at 400 mg/kg until 24 h after treatment completion. Only CTX at 400 mg/kg TID achieved levels of bacterial killing in lungs similar to those seen with CRO and AMO, 24 h after the end of treatment.

(iii) Pulmonary and blood clearance after a single drug injection in leukopenic mice infected with P40984. Lung and blood clearance values for P40984-infected mice given one injection of AMO, CTX, or CRO are shown in Table 4. With this strain, bacterial growth in the lungs was slower than with strain P40422. Four hours after a single injection, CTX at 400 mg/kg achieved a significant decrease in bacterial counts compared with controls. Eight hours after treatment, bacterial counts were significantly lower in the CTX at 400 mg/kg and CRO at 200 mg/kg groups than in all other groups. 24 hours after a single injection of CRO at 200 mg/kg, lung bacterial counts were significantly lower than in the other treatment groups with the exception of the CRO at 100 mg/kg group. At this time, bacterial counts were also significantly lower with CRO at 100 mg/kg than with CTX at 400 mg/kg ($P < 0.05$). Blood cultures were always negative with the two different doses of CRO.

Survival rates. Survival rates correlated well with lung clearance data. With strain P40422 (Fig. 1), untreated mice died within 1 to 3 days. A cumulative survival rate of 85% was obtained with CRO at 100 mg/kg BID ($P = 10^{-4}$ versus the untreated group). AMO at 200 mg/kg TID and CTX at 200 mg/kg TID achieved survival rates of only 40 and 30%, respectively ($P < 0.05$ versus CRO at 100 mg/kg). With these two drugs, a dose of 400 mg/kg was needed to protect 85 and 66% of the animals, respectively ($P = 0.09$ versus CRO at 100 mg/kg).

With the less virulent strain P40984, controls died within 2 to 4 days. Treatment with CRO at 100 mg/kg BID was associated with a survival rate of only 54%, whereas doubling the dose (200 mg/kg BID) provided good efficacy with an 83% survival rate. Dosages of AMO and CTX of 400 mg/kg TID were associated with survival rates of 66 and 75%, respectively, with no significant difference versus CRO at 200 mg/kg TID (Fig. 2).

Pharmacokinetic studies. After uninfected mice were given a single injection of AMO, peaks in serum and lungs were higher with a dose of 400 mg/kg (212 and 52.6 μg/ml, respectively) and lasted twice as long as with a dose of 200 mg/kg (Table 5). CTX at 400 mg/kg was also associated with a higher peak concentration in serum than CRO at 100 mg/kg (191 μg/ml versus 91.5 μg/ml), but CRO at 100 mg/kg produced a

TABLE 3. Clearance, during multiple-dose s.c. treatment, of *S. pneumoniae* from lungs of leukopenic Swiss mice infected with strain P40422[a]

Drug and dose (mg/kg)	$\log_{10}$ CFU at time (h)[b]:			
	8	32	62	86
None (controls)	7.52 ± 0.27	8.23 ± 0.33	8.93 ± 0.3	
AMO, 400	5.80 ± 0.38[c]	4.20 ± 0.16[c,d]	2.90 ± 0.33[c,d]	<2[d]
CTX, 200	6.80 ± 0.36	7.60 ± 0.27	5.11 ± 1.49[c]	4.96 ± 0.24
CTX, 400	6.55 ± 0.33	6.64 ± 0.52[c]	5.0 ± 0.4[c]	2.82 ± 0.8[f]
CRO, 100	5.70 ± 0.38[c,d]	3.69 ± 0.25[c,d]	<2[e]	<2[c,d]

[a] Treatment was initiated 3 h after infection. Doses are per injection. The lower limit of detection was 2 $\log_{10}$ CFU per lung.
[b] Values are $\log_{10}$ CFU per milliliter of lung homogenate and are means ± standard deviations ($n = 3$).
[c] Value is lower than in the controls ($P < 0.03$).
[d] $P < 0.05$ versus CTX at 200 and 400 mg/kg.
[e] $P < 0.05$ versus all other groups.
[f] Value is lower than in the CTX at 200 mg/kg group ($P < 0.05$).

TABLE 4. Clearance, after a single s.c. injection of study drug, of *S. pneumoniae* from lungs and blood of leukopenic Swiss mice infected with P40984

Drug and dose (mg/kg)	Clearance after a single s.c. injection as determined at time (h)[a]:							
	1		4		8		24	
	From lung	From blood	From lung	From blood	From lung	From blood	From lung	From blood
None (controls)	6.63 ± 0.24	2/4	6.41 ± 0.08	3/4	6.90 ± 0.64	3/4	7.40 ± 0.56	4/4
AMO 400	6.87 ± 0.19	2/4	6.49 ± 0.22	0/4	5.99 ± 0.36	3/4	5.32 ± 0.74[b]	1/4
CTX 400	6.41 ± 0.09	0/4	5.71 ± 0.29[b]	1/4	4.59 ± 0.52[b]	1/4	5.38 ± 1[b]	1/4
CRO 100	6.56 ± 0.21	0/4	6.21 ± 0.35	0/4	6.13 ± 0.27	0/4	4.08 ± 0.8[b]	0/4
CRO 200	6.60 ± 0.3	0/4	6.23 ± 0.23	0/4	5.16 ± 0.53[b]	0/4	3.80 ± 0.55[b,c]	0/4

[a] For lung, values are mean $\log_{10}$ CFU per milliliter of lung homogenate ± standard deviations ($n = 4$). For blood, values are the number of animals with positive blood cultures/total number of animals.
[b] Value is lower than in the control group ($P < 0.05$).
[c] Value is lower than in the other treatment groups ($P < 0.05$).

higher peak concentration in the lungs than CTX, even when the latter was at a dose of 400 mg/kg (44.3 μg/g versus 25.1 μg/g); the value of the peak with CRO at 100 mg/kg was similar to that seen with AMO at 400 mg/kg. The elimination half-life in serum was longer with CRO than with AMO or CTX (1.35 h versus 0.43 and 0.33 h), and the elimination half-life in the lungs was shorter with CTX than with the other study drugs. Although serum AUCs were identical with AMO at 400 mg/kg, CTX at 400 mg/kg, and CRO at 100 mg/kg (189, 183.5, and 150 μg · h/liter, respectively), the intrapulmonary AUC was smaller with CTX (19.3 μg · h/liter versus 70.3 and 59.6 μg · h/g with AMO and CRO, respectively). The Δ*t*MICs for the P40422 and P40984 strains were longer with CRO at 100 mg/kg (4.6 and 3.6 h, respectively) than with CTX at 200 mg/kg (2.6 and 1.9 h, respectively) or AMO at 200 mg/kg (3.3 and 2.5 h, respectively) (Fig. 3).

DISCUSSION

Highly penicillin-resistant (MICs, ≥2 μg/ml) and multidrug-resistant strains have emerged during the last two decades (4, 26), creating a need for alternatives to penicillin. Although the use of broad-spectrum cephalosporins, such as CRO and CTX, has been advocated, strains of pneumococci with resistance to these drugs have been identified (10, 17, 25).

Using a mouse model of pneumonia, we previously demonstrated the efficacy of CRO at a dosage of 50 mg/kg BID against a strain of *S. pneumoniae* (P15986) with high-level resistance to penicillin (MIC = 4 μg/ml for penicillin and 2 μg/ml for AMO) and low-level resistance to cephalosporins (MIC = 0.5 μg/ml) (31). CTX in a fourfold-higher dose (200 mg/kg BID) showed similar efficacy. Administration of the same daily dose of CTX (400 mg/kg) as four injections at 6-h intervals produced a survival rate of only 60% (unpublished data). The Δ*t*MIC appeared to be the main pharmacodynamic determinant of the efficacy of CRO (19, 28, 31).

In the present study, with two strains characterized by high-level resistance to penicillin and by resistance to cephalosporins, CRO at a dose/MIC ratio of 25 remained very effective. With this dose, the Δ*t*MIC was 4.6 h for the P40422 strain, explaining the clearance of bacteria from the lungs after a single injection. With injections at 12-h intervals, CRO levels were greater than the MIC for the P40422 strain during 38% of the dosing interval. This result is in accordance with a recent

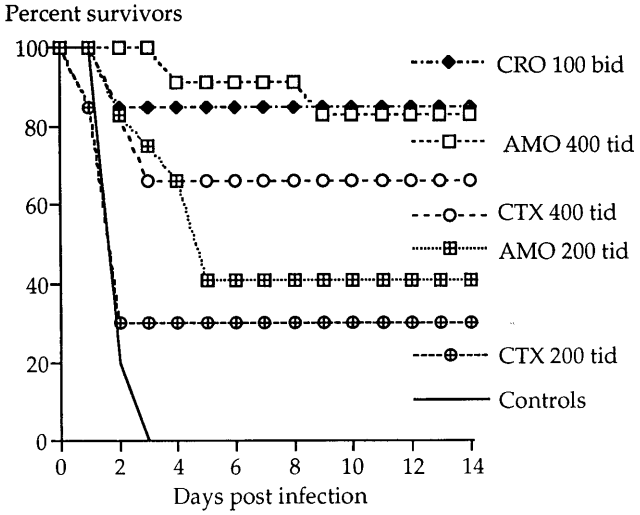

FIG. 1. Cumulative survival of treated and control leukopenic Swiss mice after intratracheal challenge with 10^7 CFU of strain P40422. Three hours postinfection, mice received s.c. injections of CRO every 12 h (BID) or AMO and CTX every 8 h (TID) at the indicated amount of each drug (in milligrams per kilogram of body weight).

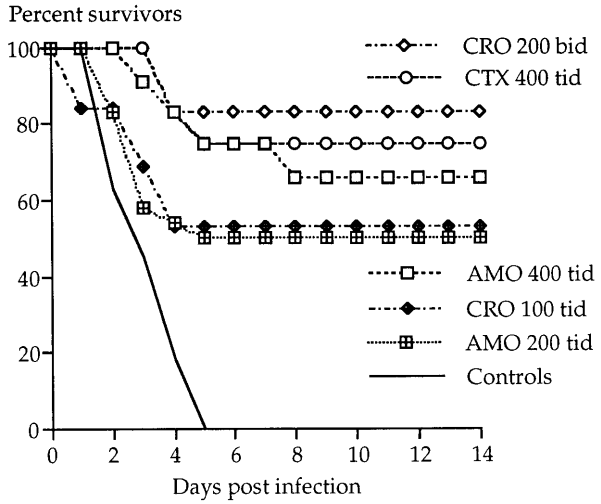

FIG. 2. Cumulative survival of treated and control leukopenic Swiss mice after intratracheal challenge with 10^7 CFU of strain P40984. Three hours postinfection, mice received s.c. injections of CRO every 12 h (BID) or AMO and CTX every 8 h (TID) at the indicated amount of each drug (in milligrams per kilogram).

TABLE 5. Pharmacokinetic parameters of AMO, CTX, and CRO in Swiss mice following a single s.c. injection[a]

Drug and dose (mg/kg)	Site	C_{max} (μg/ml or μg/g)[b]	$t_{1/2\beta}$[c]	ΔtMIC (h)[d]	AUC_{0-10} (μg · h/ml or μg · h/g)
AMO, 200	Serum	118 ± 11	0.41	3.3	92
	Lung	22.5 ± 6	1.98	3.7	32.5
AMO, 400	Serum	212 ± 38	0.43	3.8	189
	Lung	52.6 ± 15	1.94	5.1	70.3
CTX, 200	Serum	100.9 ± 10	0.32	2.6	94.5
	Lung	13.4 ± 6	0.41	0.5	10.1
CTX, 400	Serum	191 ± 36	0.33	2.8	183.5
	Lung	25.1 ± 13	0.35	1.3	19.3
CRO, 100	Serum	91.5 ± 10	1.35	4.6	150
	Lung	44.3 ± 5	1.68	4	59.6

[a] Values are calculated from mean concentrations in serum and lung tissue samples taken at 0.5, 1, 2, 4, 6, 8, 10, and 24 h postinfection.
[b] C_{max}, maximum concentration observed. Values are expressed as means ± standard deviations (n = 3 mice).
[c] $t_{1/2\beta}$, terminal elimination half-life calculated by least-squares regression for the log-linear terminal elimination phase.
[d] Maximum residential time above the MIC calculated for strain P40422 (MIC = 2 for AMO and 4 for CTX and CRO).

study in a neutropenic-mouse thigh model, in which AMO was bactericidal when serum AMO concentrations exceeded the MIC for *S. pneumoniae* during 40 to 50% of the dosing interval (2). Moreover, there was no significant regrowth between 8 and 24 h after a single CRO dose in our study. Even with identical CRO and CTX MICs, lower 50% protective doses have been reported with CRO than with CTX in murine experimental models of pneumonia and meningitis induced by susceptible strains of *S. pneumoniae* (9). In our study, the weak bactericidal effect of high-dose CTX (200 and 400 mg/kg) at any time after a single injection reflected the shorter ΔtMIC of CTX versus that of CRO. CTX was effective, in terms of bacterial clearance and survival rates, only when given at 8-h intervals at the high dose of 400 mg/kg. With this regimen, the concentration of CTX was above the MIC for the P40422 strain during 35% of the dosing interval. With CTX at 200 mg/kg TID (32% of the dosing interval spent above the MIC), the bactericidal effect was slow and weak and the survival rate was low, suggesting a dose-effect relationship for the efficacy of CTX. Moreover, CTX produced lower pulmonary concentrations than the other study drugs. The relevance of drug-protein binding to drug efficacy is controversial (15, 30, 34, 40). As in humans (13), the affinity of CRO for proteins has been shown to be high and concentration dependent in mice (85 to 96%) (31). CRO bound to serum proteins is easily dissociated, and the bound fraction may act as a temporary reservoir of the drug (19).

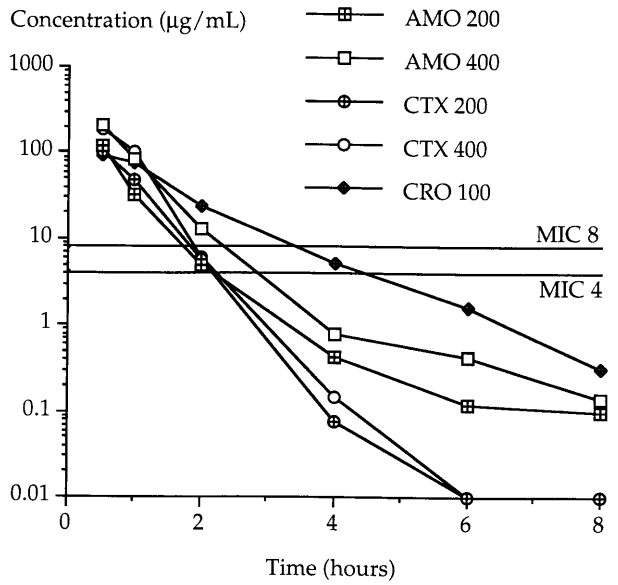

FIG. 3. Pharmacokinetic parameters of AMO, CTX, and CRO in serum after a single s.c. injection at the indicated amount of each drug (in milligrams per kilogram).

The pharmacodynamic parameters that determine the bactericidal behavior of AMO in penicillin-resistant *S. pneumoniae* strains have not been clearly identified. In a leukopenic-mouse thigh model, ΔtMIC seemed to be the main determinant of bactericidal activity of AMO against penicillin-resistant *S. pneumoniae* strains (2); in contrast, in a neutropenic-mouse pneumonia model, the bactericidal activity of CRO was correlated with the ΔtMIC, but it was the total daily dose of penicillin that was correlated with efficacy (28). In our model, despite a ΔtMIC of 3.3 h and a serum AMO concentration above the MIC for the P40422 strain during 41% of the dosing interval, AMO at 200 mg/kg TID was ineffective. On the other hand, AMO at the higher dosage of 400 mg/kg TID was as effective as CRO at 100 mg/kg BID, despite a ΔtMIC of 3.8 h; i.e., it was quite similar to that of the ineffective 200-mg/kg dose. This result is in keeping with the above-mentioned relationship between the total daily dose of AMO or penicillin and maximum efficacy on penicillin- and cephalosporin-resistant *S. pneumoniae* strains (28). The MIC of CRO for strain 40984 was twice that for strain 40422 (8 μg/ml versus 4 μg/ml); thus, it is not surprising that the effective dose of CRO has to be doubled (from 100 mg/kg to 200 mg/kg). We have no clear explanation for the better activity of AMO and CTX at a dose of 400 mg/kg on this strain compared with strain 40422, which contrasts with the need to increase the dose of CRO. At best, one might speculate that it reflects differences in the bactericidal response between strains of *S. pneumoniae* due, for example, to different tolerance or autolysis properties.

Other parameters may contribute to the differences in activity between CRO and CTX. The killing activity of β-lactam antibiotics on *S. pneumoniae* is primarily due to interactions of these agents with penicillin-binding proteins (PBP), which produce uncontrolled activity of autolysins (27, 42). Inhibition of PBP 2B is an important determinant in activation of this autolysis system. Penicillin- and cephalosporin-resistant isolates of *S. pneumoniae* exhibit specific PBP modifications, with changes in the affinities of PBPs 2B, 1A, and 2X (17, 23, 32, 41, 43, 46). Earlier studies found that CRO had greater affinity for the PBP 2Bs of various strains of *E. coli* and *Haemophilus influenzae* than other cephalosporins (particularly CTX, which

had very weak affinity for PBP 2B) and was associated with the highest rates of lysis of these strains (22, 44). Further studies of PBP binding of CRO and CTX and of the lysis-inducing activity of these agents on penicillin- and cephalosporin-resistant *S. pneumoniae* strains are needed.

In conclusion, CRO showed good efficacy against *S. pneumoniae* strains with high-level cephalosporin resistance in a leukopenic-mouse pneumonia model. Despite their similar MICs, CTX was less active than CRO. The favorable pharmacokinetic properties of CRO accounted in large part for its efficacy being greater than that of CTX.

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